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14. ABSTRACT The award is transferred to the PI 10/1/08. During this short period, we identified a novel androgen responsive element in the promoter region of cyclinB1 that negatively regulates its expression in AR-positive prostate stromal cells. Cyclin B1 expression is maintained by E2F1 in prostate stromal cells in the absence of androgen. Upon stimulation with androgen, E2F1 is displaced from the promoter by E2F4, leading to recruitment of the SMRT co-repressor complex, and repression of cyclin B1 expression. These results strongly indicate cyclin B1 as a bona fide AR target gene negatively controlled by androgen-dependent E2F regulation. Next, we will determine the association between stromal cyclinB1 expression and prostate cancer racial disparity.

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No subject terms provided

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INTRODUCTION

AR mediates transcriptional activation through a series of events including ligand binding, binding to cognate androgen response elements (AREs) and interaction with various coactivators, resulting in transcriptional initiation of AR target genes by the general transcriptional machinery. The mechanism responsible for the switch of AR mediated growth promotion and inhibition could be due to the different set of AR target genes activated. Recent efforts in identifying AR target genes in prostatic cell lines and human cancer using DNA microarrays have resulted in the identification of several AR target genes. It is of particular interest of this proposal to determine whether any of these are also expressed in prostate stromal cells and whether their expression contributes to racial disparity of prostate cancer. We have characterized cyclinB1 as a novel negatively regulated AR target gene. Next, we will determine whether stromal cyclinB1 is associated with prostate cancer racial disparity.

BODY:

Identification of a novel ARE negatively regulating the transcription of *cyclin B1*

Our findings indicated that in the presence of androgen, androgen receptor mediated the repression of cyclin B1 transcription. Androgen receptor activates or represses transcription through binding to specific DNA sequences termed androgen response elements (AREs) in promoter regions of AR target genes. To examine whether the androgen receptor mediated transcriptional repression of cyclinB1 is directed through ARE(s) on the cyclinB1 promoter, we first performed ChIP assays with anti-AR antibody to immunoprecipitate the protein-DNA complex and used primers specific for the *cyclinB1* promoter to detect its presence in the complex. The results showed that AR recruitment to the promoter region of cyclinB1 was enhanced in the presence of androgen. Further, the promoter occupancy of AR on cyclinB1 promoter is confirmed by reporter ChIP assays with reporter plasmid containing 1kb of the *cyclinB1* proximal promoter transiently transfected into PShTertAR cells. Lastly, dual luciferase assays with pcycB.1kb-LUC bearing the 1kb *cyclin B1* proximal promoter showed transcriptional repression by AR in the presence of androgen. These results strongly support the direct occupancy of AR at the *cyclin B1* promoter.

To delineate potential AREs, we first established various *cyclin B1* promoter-luciferase reporter plasmids with 200bp serial deletions of the 1 kb proximal promoter region. All constructs resulted in equal levels of transcription repression, showing the region was located in the 200bp proximal promoter construct. The 200bp construct was further dissected into a 70 bp proximal fragment (70bp-LUC) and a 130 bp distal fragment (130bp-LUC). Luciferase assays indicated that the 130bp-LUC luciferase reporter lost inducibility upon treatment with R1881, suggesting that the potential ARE is located in the 70 bp region. To confirm that the potential ARE is located in 70 bp region, we performed reporter ChIP with the 200 bp-LUC, 130bp-LUC and 70bp-LUC luciferase reporter plasmids. As expected, both 200 bp-LUC and 70 bp-LUC reporters gave positive PCR bands while no band was detected for the 130 bp-LUC reporter, indicating that the potential ARE is in the 70 bp region. Inspection of the 70 bp DNA sequence with known AREs suggested that the 16 bp region (from +15 to +31) resembles the AREs of the p21 gene. To further verify that this consensus sequence represents an authentic ARE, site-directed mutations were introduced in this region in the 200bp-LUC reporter. The mutant 200bp-LUC reporter was not repressed by androgen, indicating the 16bp region (from +15 to +31) in the cyclinB1 promoter is a bona fide ARE.

KEY RESEARCH ACCOMPLISHMENTS

Identification of a novel ARE negatively regulating the transcription of *cyclin B1*

REPORTABLE OUTCOMES

1. Li Y, Zou X, **Daniels G**, Ye H, Melamed J, Ouyang JY, Pellicer A, Chiriboga L, Pagano M, Peng Y, Garabedian MJ, Dynlacht B, Lee P Switch between E2F4 and E2F1 in regulation of novel AR target gene cyclinB1 in prostate stromal cells (submitted)

CONCLUSION

We have identified cyclinB1 as a novel negatively regulated AR target gene in prostate stromal cells.